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DRDE REPORT

Acute Oral Toxicity Studies of Samples of "Stored Toxic Wastes at the former UCIL Plant Site at Bhopal" in Rats



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Performing Laboratory

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MANAGEMENT STATEMENT

This is to certify that the research entitled "Acute Oral Toxicity Studies of Samples of 'Stored Toxic Wastes at the former UCIL Plant Site at Bhopal' in Rats " was carried out by the Animal Facility Division and Pharmacology and Toxicology Division, Defence Research and Development Establishment, Jhansi Road, Gwalior, M.P., India. This report contains 30 pages including front page, contents and tables.

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LIST OF ABBREVIATIONS

ALP	-	Alkaline phosphatase
bw	-	Body weight
dl or dL	-	Decilitre
F	-	Female
g	-	Gram
g/kg bw	-	Gram per kilogram of body weight
Hb	-	Haemoglobin
HPC	-	Hydroxypropyl cellulose (1% w/v in distilled water)
IU/L	-	International unit per litre
L	-	Litre
M	-	Male
mg	-	Milligram
N	-	Number of observations (total)
NAD	-	No abnormalities detected
No. or N°	-	Number
OBI	-	Organ - Body Weight Index
%	-	Per cent
q.s. (or Q.S.)	-	<i>quan'tum sa'tis</i> (L.) (a sufficient amount)
RBC	-	Red blood cell
sec	-	Second
SE	-	Standard error of mean
SGOT	-	Serum glutamate oxaloacetate transaminase
SGPT	-	Serum glutamate pyruvate transaminase
ul or μ l	-	Microlitre
WBC	-	White blood cell

SUMMARY

This study was conducted to assess the safety with special reference to the acute toxicity, if any of six samples (Excavated waste, Lime sludge, Naphthol tar (or Napthol tar), Reactor residue, Semi processed pesticide and Sevin tar) collected from 'Stored Toxic Wastes at the former UCIL Plant Site at Bhopal'. All the six coarse samples were separately collected in polythene bags and brought to DRDE, Gwalior. The samples were grinded, homogenized and filtered using 40 mesh sieve and stored in glass bottles at room temperature.

Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as *acute* toxicity, the adverse effects should occur within 14 days of the administration of the substance. Toxicity studies of all the chemicals/ materials are a prerequisite, before the material is allowed for human usage. This is essential for safety evaluation of the material. The rat was selected as a test system because it is a readily available laboratory rodent species. It has been historically shown to be a suitable model for toxicity assessment. The oral route represents inadvertent/ accidental route of administration specially during handling of the samples by humans.

Briefly, each of the samples was separately triturated using ceramic mortar and pestle in 1% hydroxylpropyl cellulose (HPC in distilled water). The required volume (not more than 1% of body weight) of freshly prepared suspension was fed orally to the rats using oral dosing needle. Three doses of each sample viz. 0.80, 1.60 and 3.20 g/kg of body weight to male rats and two doses (1.60 and 3.20 g/kg) to female rats (n=4 for each dose) were fed. The rats were observed for 14 days for development of any toxic symptoms and also for mortality.

Results: The median lethal dose (LD_{50}) of the samples calculated following the moving average method of Gad and Weil (1989), considering that a dose of 6.40 g/kg body weight will kill all the rats, are as follow: (i) Excavated waste 4.525 g/kg in both sexes; (ii) Lime sludge 4.525 g/kg in both sexes; (iii) Naphthol tar male rats 3.80 g/kg and female rats 4.525 g/kg; (iv) Reactor residue 4.525 g/kg in both sexes; (v) Semi processed pesticide 4.525 g/kg in both sexes and (vi) Sevin tar male rats 2.69 g/kg and female rats 3.20 g/kg.

The important finding of the study: Oral LD_{50} of five samples i.e. Excavated waste, Lime sludge, Reactor residue, Semi processed pesticide and Naphthol tar (in female rats) were found to be more than 4.5 g/kg. Sevin tar indicated more toxicity compared to other five samples. However, this can not be considered as highly toxic compound as LD_{50} was 2.69 g/kg or more.

Conclusion

All the samples viz. Excavated waste, Lime sludge, Naphthol tar, Reactor residue, Semi processed pesticide and Sevin tar were found to be having very low mammalian toxicity.

INTRODUCTION

This study was conducted to assess the safety with special reference to the acute toxicity of samples collected from 'Stored Toxic Wastes Lying at the former UCIL Plant Site at Bhopal'. The samples collected were as follow:

- (i) Excavated waste,
- (ii) Lime sludge,
- (iii) Napthol tar,
- (iv) Reactor residue,
- (v) Semi processed pesticide and
- (vi) Sevin tar

All the six coarse samples were separately collected in polythene bags and brought to Defence Research and Development Establishment (DRDE), Gwalior for the aforesaid study.

Significance of acute toxicity studies

Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as *acute* toxicity, the adverse effects should occur within 14 days of the administration of the substance. Toxicity studies of all the chemicals/materials is a prerequisite, before the material is allowed for human usage. This is also essential for safety evaluation of the material. The toxicity tests have to be carried out in laboratory animals using the possible routes of entry of chemicals viz., oral, dermal etc. The results thus obtained are believed to be of value in predicting acute toxicity, if any of the samples in humans.

The rat was selected as a test system because it is a readily available laboratory rodent species. It has been historically shown to be a suitable model for toxicity assessment. The oral route represents inadvertent/accidental route of administration specially during handling of the samples by humans.

The study was performed at Animal Facility Division and Pharmacology and Toxicology Division, Defence Research & Development Establishment, Jhansi Road, Gwalior (M.P.).

EXPERIMENTAL PROCEDURE

Test Substance

Six samples i.e. Excavated waste, Lime sludge, Napthol tar, Reactor residue, Semi processed pesticide and Sevin tar were collected from 'Stored Toxic Wastes Lying at the former UCIL Plant Site at Bhopal'. All the six coarse samples were collected in polythene bags and brought to DRDE, Gwalior. The samples were grinded, homogenized and filtered using 40 mesh sieve and stored in glass bottles at room temperature. After grinding and filtering all the samples became as free flowing dry powder and appearance was as follow:

Housing

The animals were housed in groups of four of same sex in solid floored polypropylene rat cages (size 430 x 290 x 150 mm). Each cage was fitted with a stainless steel top grill having provision for keeping pellet feed and a glass water bottle with stainless steel drinking nozzle. The bottom of the cage was layered with cleaned and steam autoclaved rice husk. The cages were kept on 3 tier racks and their positions were rotated from time to time.

Diet and Water

The animals were provided *ad libitum* laboratory rat pellet feed (Ashirwad brand, Chandigarh, India) and potable tap water. Fresh pellet feed was supplied and water bottles refilled daily.

Grouping

The animals were randomly allocated to seven different groups. The animals were equally distributed (for each group 4 rats per cage) to seven groups for each dose (three doses i.e. 800, 1600 and 3200 mg/kg of body weight, oral) viz., (i) Excavated waste, (ii) Lime sludge, (iii) Naphthol tar, (iv) Reactor residue, (v) Semi processed pesticide (vi) Sevin tar and (vii) Hydroxypropyl cellulose (HPC) 1 % or control (total $7 \times 3 = 21$ groups for male rats and $7 \times 2 = 14$ groups for female rats).

Animal Identification

Individual animal was identified with picric acid (aqueous 1.2% w/v) over the body coat, and coloured cage label showing Study No. and Sex, Dose, Cage No. and Animal No. etc.

Route and Mode of Administration

Each of the samples was separately triturated using ceramic mortar and pestle in 1% hydroxylpropyl cellulose (HPC in distilled water). The required volume (not more than 1% of body weight) of freshly prepared suspension was fed orally to the rats using oral dosing needle.

Determination of Median Lethal Dose (LD₅₀)

LD₅₀ following oral route of administration was determined in male and female rats keeping the observation period for mortality 14 days following the method described by Gad and Weil, 1989.

Duration of Treatment and Observations

The animals were treated once and observed for 14 days for gross behavioural changes e.g. itching, restlessness and mortality. And sacrificed on 14th day for estimation of various haematological and clinical biochemical variables.

Experimental Outline

	Group	Dose (mg/kg of body weight) Oral	Number of rats (Males + Females)			
			Treated		Clinical Pathology	
			M	F	M	F
1.	Excavated waste	800	4	-	4	-
		1600	4	4	4	4
		3200	4	4	4	4
2.	Lime sludge	800	4	-	4	-
		1600	4	4	4	4
		3200	4	4	4	4
3.	Naphthol tar	800	4	-	4	-
		1600	4	4	4	4
		3200	4	4	4	4
4.	Reactor residue	800	4	-	4	-
		1600	4	4	4	4
		3200	4	4	4	4
5.	Semi processed pesticide	800	4	-	4	-
		1600	4	4	4	4
		3200	4	4	4	4
6.	Sevin tar	800	4	-	4	-
		1600	4	4	4	4
		3200	4	4	4	4
7.	HPC 1 % w/v in distilled water or control	Equal vol.	4	-	4	-
		Equal vol.	4	4	4	4
		Equal vol.	4	4	4	4

Body Weight

Individual body weight was recorded for all the animals for 14 days.

Stability of Test Substance in ready to use Solution

Freshly prepared solutions of all the samples were used.

Observations

Clinical Signs

Animals were observed for mortality and morbidity at least twice a day. All gross visible signs and symptoms such as skin and/or fur changes, eye and mucous membrane changes, respiratory, autonomic nervous system, somato-motor activity, behavioural pattern and general changes were recorded once a day.

Clinical Pathological Observations

Clinical pathological tests were conducted on blood collected from all the animals after 14 days. The control group of the animals were also sacrificed after 14 days. Animals were deprived of food overnight prior to blood collection and blood samples were collected by puncturing the orbital sinus plexus with the help of a fine glass capillary tube under light ether (anaesthetic) anaesthesia.

About 0.5 ml blood was collected in vials containing heparin for haematological analysis. All the undermentioned haematological variables were examined using Beckman Coulter Cell Counter (Model Ac.T. DiffTM Analyzer).

Three to four ml of blood was collected from each animal in clean centrifuge tubes for plasma separation. The plasma was separated by centrifugation at low speed at room temperature. The plasma thus separated was kept in cold condition and subjected to the studies. Haematological and clinical biochemistry parameters and instrument used are given below:

Haematology

Parameter	Sample Type	Instrument used
Total WBC ($\times 10^3 \mu\text{l}$) count	Whole blood	Beckman Coulter Cell Counter
Total RBC ($\times 10^6 \mu\text{l}$) count	Whole blood	Beckman Coulter Cell Counter
Platelets ($\times 10^3 \mu\text{l}$)	Whole blood	Beckman Coulter Cell Counter
Haemoglobin (g/dl)	Whole blood	Beckman Coulter Cell Counter
Haematocrit (%)	Whole blood	Beckman Coulter Cell Counter

Clinical biochemistry

Parameter	Sample Type	Instrument used
Glucose (mg/dl)	Plasma	Semi-Auto Analyser *
SGPT (IU/dl)	Plasma	Semi-Auto Analyser
SGOT (IU/dl)	Plasma	Semi-Auto Analyser
ALP (IU/dl)	Plasma	Semi-Auto Analyser
Protein (g/dl)	Plasma	Semi-Auto Analyser
Cholesterol (mg/dl)	Plasma	Semi-Auto Analyser
Urea (mg/dl)	Plasma	Semi-Auto Analyser
Creatinine (mg/dl)	Plasma	Semi-Auto Analyser
Bilirubin (mg/dl)	Plasma	Semi-Auto Analyser
Albumin (g/dl)	Plasma	Semi-Auto Analyser

* Diagnostic kits of Merk (India) Ltd., Mumbai and using Semi-Auto Analyser - ALFA WASSERMANN, Model - PLD-951.

Organ-Body Weight Indices and preservation of organs for histopathology

All the animals were euthanized by cervical dislocation under light ether (anaesthetic) anaesthesia and subjected to a careful examination for external abnormalities before the necropsy. The thoracic, abdominal and cranial cavities were cut open and thorough examinations of the organs were carried out to detect changes of abnormalities, if any. Absolute weights of the organs i.e. lungs, liver, kidneys, spleen, testis/ovaries and heart were recorded immediately after dissection of all animals at the end of the study period. Paired organs were weighed together. Body-Organ weight Indices (BOI) were calculated later.

The following organs were collected from all the animals at necropsy and preserved in appropriate fixative (testis and ovaries were fixed in Bouin's solution and rest of the organs in 10% buffered formalin solution) for processing and microscopic examination (if required). However, the excised lungs with trachea were inflated *in situ* with buffered formalin (pH 7.2) solution through a tracheal cannula to an intrapulmonary pressure of 10 cm water. The whole lung was then immersed in the fixative.

Determination of Median Lethal Dose (LD₅₀)

The LD₅₀ values of all the six samples including HPC were determined in male and female rats following oral route of administration. The observation period for mortality of the treated animals was 14 days. The dilution of the samples was so done that the feeding volume was approximately 1% of the body weight. The LD₅₀ determinations were done by the moving average method of Gad and Weil (1989) using two to three groups, each group consisting of four animals.

Protection Studies

Keeping the biological activity of the parent compound i.e. Sevin in view, antidotal efficacy of atropine sulfate against Sevin tar was also evaluated. Briefly, 3200 mg/kg of Sevin tar was fed to the rats and within 10 minutes, atropine sulfate (10 mg/kg) was injected intraperitoneally and rats were observed for 14 days for mortality.

Evaluation of Results

The raw data were processed to calculate group means and corresponding standard error (SE) with significance between the controls and treated groups using SigmaStat, Version 1.0 (Jandel Corporation, USA) statistical software. All the data parameters characterized by continuous data such as body weight, feed consumption, organ weight, relative organ weight, haematological and clinical chemistry data were subjected to Analysis of Variance (ANOVA) and Dunnett's t-test. Significance was calculated at 5% ($p < 0.05$) level in the summary tables i.e. significantly different than control ($p \leq 0.05$).

RESULTS AND DISCUSSION

Mortality

No mortality was observed in any of male rats treated with lower doses of (800 and 1600 mg/kg) Excavated waste, Lime sludge, Naphthol tar, Reactor residue, Semi processed pesticide, Sevin tar and HPC (1%, w/v). However, in higher dose (3200 mg/kg) treated male rats, one died in Naphthol tar and three rats died in Sevin tar treated groups (Table 1). Keeping these results in view, female rats were treated with only two doses i.e. 1600 and 3200 mg/kg. The results indicated that one rat (male) died in naphthol tar (3200 mg/kg) group, three male and two female rats died in sevin tar (3200 mg/kg) groups (Table 1).

Clinical Observations

No noticeable clinical signs were observed during the experimental period in the rats treated with Excavated waste, Reactor residue, Semi processed pesticide and HPC. Rats treated with Lime sludge and Naphthol tar (3200 mg/kg) became sluggish for about 4 hours, and one rat died in Naphthol tar treated group. However, animals treated with lower dose of Sevin tar (1600 mg/kg) showed mild tremors and salivation after 15 minutes of oral administration for 5-6 hours. Higher dose of Sevin tar i.e. 3200 mg/kg administration caused severe tremors, profuse salivation, micturition and lachrymation with somato-motor sluggishness, indicating cholinergic hyperactivity, and three rats died within 24 hours (Table 2A and Table 2B). All the aforesaid signs and symptoms disappeared after 24 hours in the surviving one rat.

Body Weight

No significant variations were observed in mean body weight (% change or % increase) of Excavated waste, Lime sludge, Naphthol tar, Reactor residue, Semi processed pesticide and Sevin tar treated animals of both the sexes at lower doses (800 and 1600 mg/kg) on 14th day compared to that of control (HPC) group. However, in the higher dose (3200 mg/kg), a decrease in body weight was observed in Lime sludge and Sevin tar groups (Table 3A and 3B).

Median Lethal Dose (LD₅₀)

The LD₅₀ values of all the samples were determined assuming that double the dose (6400 mg/kg, oral) than the used highest dose (3200 mg/kg, oral), administration of all the samples killed all the four rats of all the groups. The calculated LD₅₀ of the samples were as follow: Excavated waste, Lime sludge, Reactor residue and Semi processed pesticide (male and female rats), 4.525 mg/kg. Naphthol tar 3.80 (2.48-5.81) and 4.525 mg/kg in male and female rats respectively. Sevin tar, male rats 2.69 (1.81-3.98) and female rats 3.20 (1.96-5.22) mg/kg (Table 4).

Haematology

Haematological analysis performed at the end of treatment period did not reveal treatment related changes of clinical importance for all the six compounds as compared to that of control group of male and female rats (Table 5A and 5B).

Clinical Biochemistry

Clinical biochemical analysis performed at the end of treatment period did not reveal treatment related changes of clinical importance for all the six compounds as compared to that of control group of male and female rats. However, changes in bilirubin were observed in male rats (3200 mg/kg) but of no clinical significance (Table 6A, 6B, 7A and 7B).

Relative Organ Weight/ Organ-Body Weight Indices (OBI)

No changes of clinical significance were observed in the relative organ weight in any of the groups of male and female animals sacrificed at the end of treatment period (Table 8A and 8B).

Pathological Findings

External

External examination of carcasses of either sex belonging to control as well as various treated groups did not reveal any lesion of pathological significance.

**Internal
Male rats**

Visceral examination of carcasses belonging to control and treatment groups showed no noticeable lesion in any of the organs.

Female rats

Visceral examination of carcasses belonging to control and treatment groups showed no noticeable lesion in any of the organs.

CONCLUSION

All the samples viz. Excavated waste, Lime sludge, Naphthol tar, Reactor residue, Semi processed pesticide and Sevin tar were found to be having very low mammalian toxicity.

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Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

TABLE 1

Fourteen days Mortality Record

Group	Dose (mg/kg of b. wt.)	Male (n=84)	Female (n=56)
Excavated waste	800	0/4	-
	1600	0/4	0/4
	3200	0/4	0/4
Lime sludge	800	0/4	-
	1600	0/4	0/4
	3200	0/4	0/4
Naphthol tar	800	0/4	-
	1600	0/4	0/4
	3200	1/4	0/4
Reactor residue	800	0/4	-
	1600	0/4	0/4
	3200	0/4	0/4
Semi processed pesticide	800	0/4	-
	1600	0/4	0/4
	3200	0/4	0/4
Sevin tar	800	0/4	-
	1600	0/4	0/4
	3200	3/4	2/4
Control (HPC 1% w/v)	Equal volume	0/4	-
	Equal volume	0/4	0/4
	Equal volume	0/4	0/4

Key: Died/Treated. n = Number of animals. HPC = Hydroxypropyl cellulose.

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

TABLE 2 A

Summary of Clinical Signs

Gross clinical signs:

Group	Dose (mg/kg of b. wt.)	Post Dosing Days					
		Male			Female		
		1	7	14	1	7	14
Excavated waste	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Lime sludge	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	4/4	0/4	0/4	0/4	0/4	0/4
Naphthol tar	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/3	0/3	0/4	0/4	0/4
Reactor residue	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Semi processed pesticide	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Sevin tar	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	4/4	0/1	0/1	4/4	0/2	0/2
Control (HPC 1% w/v)	Equal volume	0/4	0/4	0/4	ND	-	-
	Equal volume	0/4	0/4	0/4	0/4	0/4	0/4
	Equal volume	0/4	0/4	0/4	0/4	0/4	0/4

Score: Clinical Signs/ Number of animals. 0 (zero) = No Clinical sign. ND=Not done.
Number of observations with the sign at least once during interval.

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

TABLE 2 B

Summary of Clinical Signs

Clinical signs viz. severe tremors, profuse salivation, micturation, severe tremors, profuse salivation, micturation and lachrymation with somato-motor sluggishness, indicating cholinergic hyperactivity and lachrymation with somato-motor sluggishness, indicating cholinergic hyperactivity diarrhea etc.:

Group	Dose (mg/kg of b. wt.)	Post Dosing Days					
		Male			Female		
		1	7	14	1	7	14
Excavated waste	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Lime sludge	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Naphthol tar	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/3	0/3	0/4	0/4	0/4
Reactor residue	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Semi processed pesticide	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	0/4	0/4	0/4	0/4	0/4	0/4
Sevin tar	800	0/4	0/4	0/4	ND	-	-
	1600	0/4	0/4	0/4	0/4	0/4	0/4
	3200	4/4	0/1	0/1	4/4	0/2	0/2
Control (HPC 1% w/v)	Equal volume	0/4	0/4	0/4	ND	-	-
	Equal volume	0/4	0/4	0/4	0/4	0/4	0/4
	Equal volume	0/4	0/4	0/4	0/4	0/4	0/4

Score: Clinical Signs/ Number of animals. 0 (zero) = No Clinical sign. ND=Not done.
Number of observations with the sign at least once during interval.

Acute Oral toxicity Studies of 'Stored Toxic Wastes
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TABLE 3A

Summary of Body weight (% change) – Male Rats

Group	Dose (mg/kg of b. wt.)	Days after exposure				
		0	3	7	10	14
Excavated waste	800	100	96.0±11.8	108.2±1.52	113.5±1.04	113.2±2.10
	1600	100	102.6±3.28	109.3±3.32	113.5±3.10	109.4±2.78
	3200	100	100.6±2.57	101.4±3.61	107.1±4.03	110.6±4.97
Lime sludge	800	100	104.4±1.76	110.6±3.85	117.3±5.21	118.4±6.81
	1600	100	103.3±3.30	111.6±4.17	117.2±3.22	118.0±2.88
	3200	100	85.2±2.79	88.05±5.75	92.97±6.53	96.7±7.09
Naphthol tar	800	100	107.4±1.11	109.2±2.06	116.5±2.37	121.1±2.87
	1600	100	105.5±0.76	108.5±0.76	109.9±2.01	111.6±2.69
	3200	100	102.9±3.62	104.8±6.84	109.1±7.21	111.2±5.72
Reactor residue	800	100	108.4±0.85	113.1±0.81	117.9±2.66	121.7±3.26
	1600	100	106.5±1.21	110.7±3.08	112.6±3.64	112.9±5.20
	3200	100	102.2±0.86	108.1±3.10	111.5±3.83	112.5±4.16
Semi processed pesticide	800	100	106.1±1.96	109.5±1.96	112.2±5.19	114.1±5.42
	1600	100	106.5±2.64	110.6±2.89	112.8±3.50	115.7±4.92
	3200	100	100.0±2.33	102.1±3.04	106.4±4.10	106.4±6.45
Sevin tar	800	100	102.7±0.70	105.8±1.35	110.0±1.95	108.9±0.35
	1600	100	99.3±1.72	107.6±2.24	109.7±2.35	110.7±2.37
	3200	100	80.86±0.00	96.5±0.00	102.1±0.00	109.5±0.00
Control (HPC 1% w/v)	Equal volume	100	105.8±2.15	114.6±1.08	117.4±1.77	120.3±2.24
	Equal volume	100	107.6±0.44	113.5±2.08	114.7±1.96	116.0±2.36
	Equal volume	100	103.7±1.70	109.1±3.11	111.8±3.58	115.5±4.76

Values are ± SE; Number of animals = 4.

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

TABLE 3B

Summary of Body weight (% change) –Female Rats

Group	Dose (mg/kg of b. wt.)	Days after exposure				
		1	3	7	10	14
Excavated waste	800	-	-	-	-	-
	1600	100	99.5±0.94	100.8±1.28	101.8±1.34	96.7±2.19
	3200	100	105.5±0.57	107.8±0.80	108.6±1.33	101.1±0.58
Lime sludge	800	-	-	-	-	-
	1600	100	99.5±0.36	97.2±0.70	96.6±0.68	92.8±1.96
	3200	100	105.2±0.87	106.8±1.02	105.5±1.25	100.4±1.46
Naphthol tar	800	-	-	-	-	-
	1600	100	102.3±0.23	98.6±0.38	98.8±1.08	93.55±1.25
	3200	100	104.2±0.13	105.6±1.13	104.6±2.05	98.0±2.75
Reactor residue	800	-	-	-	-	-
	1600	100	101.0±1.3	97.5±2.07	99.5±2.01	95.1±1.24
	3200	100	103.4±1.64	100.7±2.31	106.2±2.52	98.5±2.09
Semi processed pesticide	800	-	-	-	-	-
	1600	100	96.1±1.85	94.1±1.72	94.7±2.25	89.8±7.21
	3200	100	104.9±0.57	105.0±1.65	106.7±1.69	98.5±1.08
Sevin tar	800	-	-	-	-	-
	1600	100	92.4±4.00	95.7±2.68	99.5±2.70	94.9±2.52
	3200	100	85.0±0.45	88.7±3.19	91.96±4.78	87.3±9.06
Control (HPC 1% w/v)	Equal volume	-	-	-	-	-
	Equal volume	100	101.7±0.92	98.9±1.51	96.7±4.80	98.7±1.20
	Equal volume	100	103.2±3.87	106.6±3.87	107.5±2.98	100.8±2.04

Values are ± SE; Number of animals = 4.

Median Lethal Dose (LD₅₀) of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

TABLE 4

Fourteen days Mortality

Group	Dose (mg/kg of b. wt.)	Male (n=84)	LD ₅₀ * (g/kg)	Female (n=56)	LD ₅₀ * (g/kg)
Excavated waste	800	0/4	4.525	-	4.525
	1600	0/4		0/4	
	3200	0/4		0/4	
Lime sludge	800	0/4	4.525	-	4.525
	1600	0/4		0/4	
	3200	0/4		0/4	
Naphthol tar	800	0/4	3.80 (2.48-5.81)	-	4.525
	1600	0/4		0/4	
	3200	1/4		0/4	
Reactor residue	800	0/4	4.525	-	4.525
	1600	0/4		0/4	
	3200	0/4		0/4	
Semi processed pesticide	800	0/4	4.525	-	4.525
	1600	0/4		0/4	
	3200	0/4		0/4	
Sevin tar	800	0/4	2.69 (1.81-3.98)	-	3.20 (1.96-5.22)
	1600	0/4		0/4	
	3200	3/4		2/4	
Control (HPC 1% w/v)	Equal volume	0/4	Not applicable	-	Not applicable
	Equal volume	0/4		0/4	
	Equal volume	0/4		0/4	

Key: Died/Treated. n = Number of animals. HPC = Hydroxypropyl cellulose.
It is assumed that at the dose of 6400 mg/kg, oral, all the rats died. * Gad and Weil, 1989.

Acute Oral toxicity Studies of 'Stored Toxic Wastes
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Table 5A

Effect on Various hematological parameters following single oral administration of various compounds in male rats

Groups	WBC $\times 10^3/\mu\text{l}$	RBC $\times 10^6/\mu\text{l}$	Hb g/dl	Haematocrit %	Platelets $\times 10^3/\mu\text{l}$
Control	24.0 \pm 1.6	7.6 \pm 0.1	11.9 \pm 0.4	37.2 \pm 0.9	787 \pm 57
EW(1600)	25.8 \pm 1.7	7.1 \pm 1.1	11.3 \pm 0.1	37.7 \pm 0.9	711 \pm 91
EW(3200)	21.6 \pm 4.1	8.0 \pm 0.4	13.1 \pm 0.4	40.7 \pm 1.9	758 \pm 82
LS(1600)	24.1 \pm 5.3	9.3 \pm 0.2	12.9 \pm 0.4	40.7 \pm 1.2	972 \pm 121
LS(3200)	26.4 \pm 4.4	8.1 \pm 0.1	12.9 \pm 0.5	41.2 \pm 1.1	603 \pm 90
NT (1600)	14.3 \pm 1.6	10.0 \pm 1.0*	13.7 \pm 1.8	44.4 \pm 5.8	836 \pm 53
NT (3200)	15.4 \pm 1.9	8.0 \pm 0.2	14.2 \pm 0.2	43.7 \pm 0.4	669 \pm 35
RR(1600)	30.4 \pm 5.5	8.3 \pm 0.2	13.0 \pm 0.3	38.3 \pm 1.6	886 \pm 92
RR(3200)	24.7 \pm 1.4	8.1 \pm 0.1	13.4 \pm 0.4	42.0 \pm 1.5	777 \pm 22
SP(1600)	24.2 \pm 1.6	9.2 \pm 0.9	12.5 \pm 0.4	39.4 \pm 2.6	1036 \pm 33
SP (3200)	23.9 \pm 5.2	7.7 \pm 0.1	11.0 \pm 0.2	37.3 \pm 0.4	724 \pm 27
ST (1600)	18.0 \pm 2.5	7.6 \pm 0.1	12.9 \pm 0.7	37.9 \pm 2.0	884 \pm 19
ST(3200)	13.9 \pm 0	8.1 \pm 0	12.1 \pm 0	38.5 \pm 0	825 \pm 0
F	2.356	2.500	2.183	1.316	3.346
P	0.022	0.015	0.033	0.249	0.002

Values in parentheses are dose of the compounds (mg/kg). Mean \pm SE (n=4); F = Frequency; P = Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR-Reactor residue; SP-Semi Processed pesticide; ST-Sevin Tar

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Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

Table 5B

Groups	WBC $\times 10^3/\mu\text{l}$	RBC $\times 10^6/\mu\text{l}$	Hb g/dl	Hematocrit %	Platelets $\times 10^3/\mu\text{l}$
Control	14.5 \pm 0.8	8.7 \pm 1.8	13.9 \pm 0.8	39.4 \pm 2.5	945 \pm 89
EW(1600)	16.3 \pm 1.8	6.5 \pm 0.1	12.6 \pm 0.2	35.9 \pm 0.8	959 \pm 63
EW(3200)	15.5 \pm 1.3	7.7 \pm 0.1	14.4 \pm 1.0	41.0 \pm 1.7	836 \pm 35
LS(1600)	12.3 \pm 1.7	7.9 \pm 0.6	14.5 \pm 0.7	41.6 \pm 2.4	713 \pm 132
LS(3200)	12.9 \pm 2.0	7.9 \pm 0.6	14.5 \pm 1.1	41.7 \pm 3.5	776 \pm 125
NT (1600)	11.4 \pm 1.6	8.2 \pm 0.5	15.4 \pm 0.8	45.5 \pm 2.7	717 \pm 140
NT (3200)	15.9 \pm 2.0	6.7 \pm 0.1	13.2 \pm 0.4	37.6 \pm 1.0	898 \pm 72
RR(1600)	12.6 \pm 0.8	7.7 \pm 0.3	15.2 \pm 0.2	43.8 \pm 1.1	664 \pm 105
RR(3200)	14.2 \pm 2.4	7.2 \pm 0.1	13.7 \pm 0.6	38.3 \pm 1.5	907 \pm 71
SP(1600)	10.1 \pm 1.2	7.9 \pm 0.3	14.5 \pm 0.4	44.3 \pm 0.6	542 \pm 59
SP (3200)	16.2 \pm 2.6	6.5 \pm 0.1	12.7 \pm 0.5	33.5 \pm 2.9	860 \pm 130
ST (1600)	9.0 \pm 1.6	7.9 \pm 0.6	14.4 \pm 0.8	44.0 \pm 2.9	529 \pm 93*
ST(3200)	11.5 \pm 1.1	6.3 \pm 0	11.60 \pm 0.5	33.6 \pm 0.8	1033 \pm 53
F	1.77	0.586	1.523	2.407	2.195
P	0.087	0.840	0.158	0.019	0.032

Values in parentheses are dose of the compounds (mg/kg)

Mean \pm SE (n=4); F= Frequency; P= Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR-Reactor residue; SP-Semi Processed pesticide; ST-Sevin Tar

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

Table 6A

Effect on few related enzyme levels following single oral administration of various compounds in male rats

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Control	112±12	34±4	142±15
EW(1600)	106±6	46±5	104±1
EW(3200)	123±6	29±3	206±87
LS(1600)	98±7	39 ±1	506±104*
LS(3200)	139±4	47±8	241±14
NT (1600)	109±2	44±8	102±12
NT (3200)	121±9	57±5	431±94*
RR(1600)	80±3	42±4	105±12
RR(3200)	134±6	56±5	129±24
SP(1600)	109±11	36±5	122±9
SP (3200)	129±7	37±7	327±95*
ST (1600)	101±17	41±4	93±7
ST(3200)	97±0	40±0	201±0
F	1.401	1.121	3.34
P	0.207	0.038	0.002

Values in parentheses are dose of the compounds (mg/kg)

Mean ± SE (n=4); F= Frequency; P= Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR- Reactor residue; SP-Semi Processed pesticide; ST - Sevin Tar

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

Table 6B

Effect on few biochemical variables following single oral administration of various compounds in male rats

Groups	Bilirubin(mg/dl)	Urea(mg/dl)	Chol(mg/dl)	Protein(g/dl)	Glu(mg/dl)
Control	0.41±0.06	28.79±2.0	31.8±1.2	6.2±0.4	97.2±4.9
EW(1600)	0.53±0.03	30.08±2.5	36.4±2.6	6.9±0.1	79.9±10.2
EW(3200)	0.18±0.02*	30.7±4.0	39.4±2.8	8.0±1.1*	80.3±5.8
LS(1600)	0.62±0.07*	41.8±3.2*	42.7±3.7	7.2±0.4	80.6±4.8
LS(3200)	0.14±0.03*	30.5±1.7	53.9±12.3	7.4±0.3	100.1±2.8
NT (1600)	0.39±0.03	26.3±3.1	31.9±1.8	5.9±0.1	96.4±8.1
NT (3200)	0.23±0.05*	29.5±2.2	44.5±3.6	7.2±0.1	96.1±6.9
RR(1600)	0.96±0.06*	23.7±2.7	33.6±4.7	6.1±0.2	72.2±5.4
RR(3200)	0.29±0.0	28.7±4.5	64.3±13.7*	7.4±0.3	93.9±2.4
SP(1600)	0.48±0.03*	40.3±2.7	34.0±3.3	5.9±0.2	62.6±5.1*
SP (3200)	0.11±0*	35.9±4.1	50.0±6.2	7.2±0.3	107.5±18.8
ST (1600)	0.55±0.04	33.9±2.1	32.1±3.2	5.2±0.2	84.3±7
ST(3200)	0.89±0*	25.8±0	49.7±0	7.1±0	201.3±0*
F	46.30	3.466	2.89	4.29	19.441
P	<0.001	<0.001	0.006	<0.001	<0.001

Values in parentheses are dose of the compounds (mg/kg)
Mean ± SE (n=4); F= Frequency; P= Probability
EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR-Reactor residue; SP-Semi Processed pesticide; ST-Sevin Tar

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

Table 7A

Effect on few related enzyme levels following single oral administration of various compounds in female rats

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Control	139±8	38.±3	69±10
EW(1600)	115±12	29 ±33	132±20*
EW(3200)	115±15	293±4	123 ±21
LS(1600)	110±19	40±2	100±16
LS(3200)	134±16	48±5	118±12
NT (1600)	117±6	35±4	62±15
NT (3200)	102±9	37±4	75±15
RR(1600)	87±7	32±2	69±3
RR(3200)	102±10	40±2	108±18
SP(1600)	106±11	47±2	136±25*
SP (3200)	127±12	45±6	83±6
ST (1600)	119±7	38±2	78±13
ST(3200)	113±13	39±9	58±3
F	1.682	2.429	3.184
P	0.109	0.018	0.003

Values in parentheses are dose of the compounds (mg/kg)

Mean ± SE (n=4); F= Frequency; P= Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR- Reactor residue; SP-Semi Processed pesticide; ST- Sevin Tar

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

Table 7B

Effect on few biochemical variables following single oral administration of various compounds in female rats					
Groups	Bilirubin(mg/dl)	Urea(mg/dl)	Choll(mg/dl)	Protein(g/dl)	Gluc(mg/dl)
Control	0.47±0.07	59.7±2.7	67.9±4.4	8.6±0.3	77.1±5.2
EW(1600)	0.48±0.08	56.0±7.2*	63.9±8.7	9.6±0.3	51.6±10.4
EW(3200)	0.19±0.03	42.7±3.7	76.2±6.5	8.9±0.4	74.9±12.8
LS(1600)	0.59±0.14	75.2±3.7	62.5±4.2	10.1±0.9	70.3±7.7
LS(3200)	0.23±0.04	48.1±4.9	67.0±2.8	9.2±0.2	60.8±5.7
NT (1600)	0.74±0.11	60.2±5.8	77.9±4.4	9.9±0.6	86.3±2.1
NT (3200)	0.46±0.16	35.2±3.0*	69.1±7.0	9.0±0.5	78.8±6.5
RR(1600)	0.49±0.03	50.8±3.7	87.5±3.4	8.1±1.2	96.5±7.8
RR(3200)	0.44±0.01	42.0±2.9*	67.1±8.2	7.8±0.5	61.1±3.4
SP(1600)	0.52±0.09	48.0±1.0	86.2±2.7	8.7±0.3	106.3±17.3
SP (3200)	0.58±0.17	35.5±5.1*	66.9±9.7	8.5±0.3	66.1±3.2
ST (1600)	0.50±0.04	65.4±3.4	76.8±6.3 *	9.2±0.2	73.2±6.1
ST(3200)	0.33±0.01	30.1±3.2*	101.1±27.4	8.2±0.8	61.6±9.61
F	2.051	8.619	1.964	1.350	3.386
P	0.045	<0.001	0.056	0.231	0.002

Values in parentheses are dose of the compounds (mg/kg)

Mean ± SE (n=4); F= Frequency; P= Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR- Reactor residue ; SP-Semi Processed pesticide; ST- Sevin Tar

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Rhopar' in Rats

Table 8A

Organ to body weight ratio of vital organs followingsingle oral administration of various compounds in male rats							
Groups	Body Wt	Lung	Liver	Kidney	Spleen	Testis	Heart
Control	236±6	1.87±0.13	8.98±0.68	1.77±0.05	1.20±0.16	2.68±0.06	0.84±0.02
EW(1600)	225±7	1.24±0.08	7.36±0.18	1.57±0.10	0.98±0.12	2.80±0.04	0.86±0.04
EW(3200)	255±28	1.35±0.07	10.15±0.51	1.69±0.04	0.94±0.27	2.39±0.21	0.98±0.19
LS(1600)	209±14	1.35±0.13	7.31±0.44	1.43±0.08	0.93±0.11	2.62±0.10	0.80±0.02
LS(3200)	225±22	2.85±1.02	10.20±0.52	1.83±0.16	1.69±0.12	2.61±0.09	0.88±0.03
NT (1600)	231±9	1.27±0.05	7.71±0.56	1.67±0.01	1.12±0.13	2.84±0.15	0.85±0.05
NT (3200)	240±17	1.69±0.18	10.44±0.62	1.62±0.06	0.95±0.07	2.78±0.05	0.76±0.15
RR(1600)	224±13	1.42±0.10	7.53±0.14	1.73±0.08	0.95±0.03	2.76±0.12	0.87±0.04
RR(3200)	232±12	1.97±0.38	9.70±0.59	1.79±0.06	0.96±0.04	2.62±0.15	0.86±0.04
SP(1600)	214±13	1.80±0.19	7.29±0.54	1.63±0.19	0.99±0.14	2.88±0.10	0.83±0.08
SP (3200)	226±24	2.06±0.09	10.23±1.02	1.77±0.07	1.88±0.32	2.83±0.12	0.87±0.04
ST (1600)	241±14	1.35±0.18	7.85±0.63	1.80±0.06	0.88±0.99	2.93±0.22	0.82±0.07
ST(3200)	252±0	1.58±0.00	10.67±0.00	1.73±0.00	0.77±0.00	2.80±0.00	0.99±0.00
F	28.03	1.935	6.223	1.539	1.087	1.363	0.652
P	<0.001	0.060	<0.001	0.152	0.397	0.225	0.785

Values in parentheses are dose of the compounds (mg/kg)

Mean ± SE (n=4); F= Frequency; P= Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR- Reactor residue; SP-Semi Processed pesticide; ST- Sevin Tar

Acute Oral toxicity Studies of 'Stored Toxic Wastes
at the former UCIL Plant Site at Bhopal' in Rats

Table 8B
Organ to body weight ratio of vital organs following single oral administration of various compounds in female rats

Groups	Body Wt	Lung	Liver	Kidney	Spleen	Ovaries	Heart
Control	224±5	2.47±0.18	6.95±0.49	1.63±0.11	0.92±0.09	0.14±0.01	0.97±0.06
EW(1600)	218±6	2.51±0.32	7.60±0.34	1.86±0.15	0.80±0.09	0.12±0.00	0.73±0.02
EW(3200)	226±3	2.19±0.42	7.54±0.50	1.71±0.05	0.90±0.03	0.13±0.01	0.81±0.08
LS(1600)	214±3	2.05±0.09	7.06±0.11	1.76±0.06	0.81±0.06	0.12±0.01	0.86±0.04
LS(3200)	222±8	2.59±0.55	6.58±0.40	1.78±0.06	0.75±0.07	0.12±0.00	0.78±0.02
NT (1600)	222±6	2.12±0.14	7.70±0.51	1.83±0.03	0.84±0.10	0.14±0.00	0.84±0.03
NT (3200)	210±9	2.40±0.30	6.51±0.31	1.80±0.16	0.74±0.05	0.10±0.01	0.85±0.06
RR(1600)	227±3	1.84±0.37	6.87±0.34	1.85±0.05	0.86±0.07	0.16±0.02	0.87±0.02
RR(3200)	226±2	2.07±0.16	6.86±0.42	1.85±0.08	1.05±0.08	0.16±0.02	0.85±0.06
SP(1600)	217±12	4.48±2.51	7.41±0.74	2.19±0.39	1.07±0.03	0.17±0.00	0.90±0.06
SP (3200)	214±5	2.16±0.34	6.57±0.15	1.45±0.06	0.90±0.07	0.14±0.00	0.88±0.04
ST (1600)	224±12	1.64±0.20	7.12±0.74	1.85±0.03	0.89±0.14	0.12±0.01	0.98±0.04
ST(3200)	210±27	2.78±0.03	9.64±2.05	1.75±0.07	0.77±0.04	0.12±0.02	0.82±0.09
P	0.815	0.343	0.156	0.121	0.320	0.111	0.128

Values in parentheses are dose of the compounds (mg/kg)

Mean ± SE (n=4); F= Frequency; P= Probability

EW-Excavated Waste; LS-Lime Sludge; NT-Naphthol Tar; RR- Reactor residue ; SP-Semi Processed pesticide; ST- Sevin Tar